

Appl. No. 10/089,534
Amdt. dated October 26, 2005
Reply to Office Action of July 26, 2005

Docket No. 58009-010600

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1-12 (canceled)

Claim 13 (currently amended) A method of forming tissue on a bio-membrane including using crystalline beta-form fibroin to allow for the survival, the proliferation and the differentiation of specialized tissue cells of the human body, comprising:

- a. seeding a plurality of human tissue cells on a bio-membrane, the bio-membrane ~~including~~ crystalline beta-form fibroin being produced by:
 - dissolving degummed silk in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;
 - filtering the dissolved solution through a porous ceramic filter;
 - diluting the dissolved solution with distilled water;
 - dialyzing the diluted solution using a dialysis membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;
 - permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a bio-membrane; and
 - immersing the bio-membrane in a solution of methanol and water to make it crystalline and insoluble in water;
- b. permitting the plurality of human tissue cells to proliferate;
- c. permitting the plurality of human cells to differentiate; and
- d. forming tissue on the bio-membrane.

Claim 14 (previously presented) The method of claim 13, wherein the plurality of human tissue cells is a plurality of skin cells, a plurality of liver cells, a plurality of mesothelial cells, a plurality of astrocytes, a plurality of human skeleton osteoblasts, a plurality of tenocytes, a plurality of human tendon fibroblasts, a plurality of chondrocytes, a plurality of cells isolated

Appl. No. 10/089,534
Amdt. dated October 26, 2005
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Docket No. 58009-010600

from cartilaginous tissues, a plurality of endothelial cells of blood vessels, a plurality of steroid-secreting cells of the adrenal cortex, a plurality of smooth muscle cells of the *tunica muscularis* of the intestine and blood vessels, a plurality of squamous epithelial cells of the oral cavity, a plurality of squamous epithelial cells of the conjunctiva/cornea or a plurality of human pre-adipocytes of the white adipose connective tissue.

Claim 15 (withdrawn) A method of production of a substrate, comprising:

dissolving degummed silk in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and

immersing the membrane in a solution of methanol and water to make it crystalline and insoluble in water.

Claim 16 (previously presented) The method of claim 13, wherein the plurality of human tissue cells originate from one human body.

Claim 17 (previously presented) The method of claim 13, wherein the plurality of human tissue cells originate from a plurality of human bodies.

Claim 18 (previously presented) The method of claim 13, further comprising:

providing glucose to the plurality of human tissue cells; and

permitting the plurality of human tissue cells to consume the glucose and secrete lactic acid.

Claim 19 (previously presented) The method of claim 13, wherein the plurality of human tissue cells includes fibroblasts, and further comprising allowing the fibroblasts to secrete extracellular matrix components and precursors to collagen fibers.

Appl. No. 10/089,534
Amdt. dated October 26, 2005
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Docket No. 58009-010600

Claim 20 (previously presented) The method of claim 13, wherein the plurality of human tissue cells are keratinocytes, and further comprising permitting the keratinocytes to form epithelium.

Claim 21 (currently amended) The method of claim 13, wherein the plurality of human tissue cells includes pre-irradiated fibroblasts and keratinocytes, and further comprising allowing the cells to proliferate and function ~~with normal cytological features~~ without an occurrence of diffpoptosis

Claim 22 (previously presented) The method according to claim 13, wherein said fibroin is secreted by the *Bombyx mori* silkworm.

Claim 23 (currently amended) The method according to claim 13, wherein the bio-membrane ~~includes~~ comprises a polymer of natural origin.

Claim 24 (previously presented) The method according to claim 13, wherein the bio-membrane includes a polymer of synthetic origin.

Claim 25 (previously presented) The method according to claim 13, wherein the fibroin in the bio-membrane is present in a quantity varying from 20% to 80% in weight.

Claim 26 (withdrawn) A method of production of a substrate, comprising:

dissolving crystalline beta form fibroin in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and

Claim 27 (new) A method of forming tissue on a membrane including crystalline beta-form

Appl. No. 10/089,534
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Docket No. 58009-010600

fibroin to allow for the survival, the proliferation and the differentiation of specialized tissue cells of the human body, comprising:

- a. seeding a plurality of human tissue cells on a membrane, the membrane being produced by:
 - dissolving crystalline beta-form fibroin in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;
 - filtering the dissolved solution through a porous ceramic filter;
 - diluting the dissolved solution with distilled water;
 - dialyzing the diluted solution using a dialysis membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;
 - permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and
 - immersing the membrane in a solution of methanol and water to make it crystalline and insoluble in water;
- b. permitting the plurality of human tissue cells to proliferate;
- c. permitting the plurality of human cells to differentiate; and
- d. forming tissue on the membrane.